bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Human specimens, neuropathological evaluation, and criteria of diagnosis

EM Eliezer Masliah

Updated date: Dec 4, 2020

An abbreviated version of this protocol was published in Science Translational Medicine in Oct 2020

LRRK2 mediates microglial neurotoxicity via NFATc2 in rodent models of synucleinopathies

DOI: 10.1126/scitranslmed.aay0399

Detailed protocol

Tissue Collection

At autopsy, the brain is divided sagittally; the left hemibrain is fixed in 10% buffered formalin while the right hemibrain is serially sectioned coronally fresh and then frozen at -70^{O}C in sealed plastic bags. Tissue blocks from the right hemibrain of the midfrontal (MF), inferior parietal (IP), and superior temporal (ST) cortices, primary visual cortex in the occipital cortex (OC), hippocampus, basal ganglia, substantia nigra and cerebellum are removed and placed in 2% paraformaldehyde for subsequent thick sectioning by vibratome. In a similar way, tissue blocks adjacent to the ones described above are stored at -70^{O}C for subsequent RNA, immunoblot for biochemical analysis. Vibratome sections (40µm thick) are stored in cryoprotective medium at -20^{O}C for subsequent immunochemical studies. The rest of the right hemibrain is sliced in 3mm thick slabs and each slab is individually stored at -70^{O}C. This tissue is subsequently used for distribution among researchers that request this material for molecular or biochemical studies. The formalin-fixed left hemibrain is serially sectioned in 1 cm slices and tissue blocks from 14 brain regions described below are processed for histopathological examination by H&E, Thio-S and immunocytochemistry to phosphorylated tau, ß-amyloid, TDP-43 and α-synuclein. The remaining fixed tissue is stored in plastic bags wrapped with formalin-soaked paper towels and placed within waterproof Tupperware boxes in our storage room. Both the fixed and frozen tissue are examined grossly with evaluation of the degree of thickening of leptomeninges, atrophy of gyri, vascular narrowing, increased intracranial pressure, softening, etc. The presence, site(s), and volume of brain occupied by infarcts are noted.

Tissue Processing, Neuropathological assessment and reporting

All cases are examined by two neuropathology experts to allow assessing diagnostic reliability and discussed at clinico-pathological conference with the ADRC group. *We follow the newly revised neuropathology NIA-A criteria.* Per these guidelines, 14 brain regions will be sampled in each case. Specifically, sections of medulla (including DMV), pons (including LC), midbrain (including SN), cerebellar cortex and dentate, thalamus and subthalamic nucleus, basal ganglia at the level of anterior commissure with nucleus basalis of Meynert, hippocampus and entorhinal cortex, anterior cingulate gyrus, amygdala, middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobual, occipital cortex (primary visual cortex and visual association cortex), and white matter at the ACA, MCA, and PCA watershed. Tissues are embedded in paraffin, sectioned at 10 µm for Thioflavin-S, and 8 µm for H&E, and immunohistochemistry as previously described with antibodies to phosphorylated tau (clone AT8, 1:200, Innogenetics, Belgium), ß-amyloid (82E1; 1:1000; Millipore) and α-synuclein (1:100, Millipore).[146] When necessary we will perform immuno-staining for ubiquitin (1:500, DAKO), TDP43 (1:1000, ProSci), FUS (1:500, ProSci) and other markers of diagnostic interest. Additional blocks and sections are taken when indicated by gross abnormalities or microscopic findings.

The new NIA-AA guidelines recommend a modified version of the Thal phases of Aβ plaque accumulation adapted to a four-point scale (the "A" of the "ABC workup"). They further recommend continued use of the staging scheme for neuropathology degeneration as described originally by Braak and Braak (the "B" component of the "ABC workup") and the recommend continued use of the Consortium to Establish a Registry for Alzheimer's disease (CERAD) protocol for neuritic plaque scoring (the "C"component of the "ABC workup"). In performing these semi-quantitative assessments of the extent of AD pathology in each brain we will follow the recommended preferred staining methods of the NIA-AA. More specifically, for Aβ plaque we will use immunohistochemistry, supplemented by thioflavin-S stains. To assign a Braak stage contingent on assessing neurofibrillary tangle pathology we will employ immunohistochemistry for tau, again supplemented by thioflavin-S. Comparing the Aβ and p-tau preparations will allow us to distinguish between diffuse and neuritic plaques in assessing specimens according to CERAD protocols for neuritic plaque scoring. As detailed in the NIA-AA guidelines (see reference), following the evaluation of these special stains all cases will be characterized and reported in the recommended format for "Alzheimer disease neuropathologic change" – A, Thal phase for a beta plaques, 0, I, II, or III, B – Braak and Braak neurofibrillary tangle stage; 0, I, II, or III, and C – CERAD neuritic plaque score; 0, I, II, or III.

Again following NIA-AA guidelines, classifications of all type of LBD will fall into one of five categories; none, brain stem predominant, limbic (transitional), neocortical (diffuse) or amygdala predominant. Lewy body pathology will be evaluated using α-synuclein immunoreactivity for identification of LB's. Hippocampal sclerosis is not uncommon in large series of brains from demented patients, and has more recently been associated with TDP-43 immunoreactive inclusions in many or perhaps majority of cases. TDP-43 inclusions are also seen in about half of our FTLD cases with ubiquitin positive inclusions with or without motor neuron disease. Consequently, again in accordance with the NIA-AA guidelines, all cases of hippocampal sclerosis and FTLD will have TDP-43 immunostains performed and the results included with the neuropathologic assessment, as indicated in the draft version of the revised NAAC neuropathology data form. FTLD brains will also be stained for tau and when positive further subdivided into Corticobasal degeneration, Progressive supranucleur palsy, dementia with argyrophilic grains, and unclassified tauopathies.Cerebral vascular disease and vascular brain injury (VBI) are common co-morbidities in brains with Alzheimer's disease or other neurodegenerative conditions in the elderly. NIA-AA guidelines recommend reporting all macroscopic VBI and enumerating microvascular lesions (microinfarcts and microhemorrhages) in standard screening sections. Diffuse white matter injury as a form of VBI is also evaluated but this is admittedly a subjective judgment by the neuropathologist.

Following completion of the neuropathological analysis a written report is submitted to the family and entered in to the ADRC digital database. Also the NACC-NP form is completed and submitted to NACC.

Special Requests and Tissue Distribution

The Core receives many requests for other types of work including scientific support for projects and immunostaining with novel antibodies. The Core also provides frozen tissue for DNA extraction from brains for genetic studies. Frozen tissue also is dissected and distributed to investigators upon request, provided the ADRC committee approves. We also provide fixed and frozen tissues either in a blind fashion, identified only by specimen number, or as neuropathologically characterized AD tissue in accordance with researcher's request for tissue containing many tangles, plaques. Since tissue from dementia with LB brains, elderly controls or other types of dementia is less abundant, requests for these will be evaluated by a Tissue Review Committee). Patient confidentiality is ensured by the use of separate neuropathology core accession numbers (our XD series) and ADRC patient identification numbers. Files with both specimen numbers and clinical data are kept in a locked storage facility, and access to computerized neuropathology core date files is password protected. All tissue usage is logged into the electronic NP data system, this database also contains information as to the tissue, location, amount, distribution and data generated.

Data management and statistical analysis

The variables obtained by the Neuropathology Core, which include plaque, tangle, and LB counts, Braak stages, synaptophysin levels and brain weight among others; are entered into a computerized data base by a Neuropathology Core Technician. This temporary database is fully compatible with the ADRC main database and regularly transfers to the Data Management and Statistics Core at the ADRC. The data generated by the Neuropathology Core is available to all investigators at the ADRC through the Data Management and Statistics Core. The Neuropathology Core will collaborate closely on statistical analyses with the Data Management and Statistics Core. Data Management and Statistics Core expertise will be sought to determine power and sample size for studies in which data are compared between groups of patients with different diagnoses.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Masliah, E. (2020). Human specimens, neuropathological evaluation, and criteria of diagnosis. Bio-protocol Preprint. bio-protocol.org/prep680.
2. Kim, C., Beilina, A., Smith, N., Li, Y., Kim, M., Kumaran, R., Kaganovich, A., Mamais, A., Adame, A., Iba, M., Kwon, S., Lee, W., Shin, S., Rissman, R. A., You, S., Lee, S., Singleton, A. B., Cookson, M. R. and Masliah, E.(2020). LRRK2 mediates microglial neurotoxicity via NFATc2 in

rodent models of synucleinopathies . Science Translational Medicine 12(565). DOI: 10.1126/scitranslmed.aay0399